Aquat. Sci. 69 (2007) 11–25
1015-1621/07/010011-15
DOI 10.1007/s00027-006-0844-3

Aquatic Sciences

Research Article

Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches

Simone Zimmerli[1], Daniel Bernet[1], Patricia Burkhardt-Holm[2], Heike Schmidt-Posthaus[1], Pascal Vonlanthen[1], Thomas Wahli[1] and Helmut Segner[1,*]

[1] Centre for Fish and Wildlife Health, University of Bern, P.O. Box 8466, CH-3001 Bern, Switzerland
[2] Program Man-Society-Environment (MGU), University of Basel, Vesalgasse 1, CH-4051 Basel, Switzerland

Received: 9 November 2005; revised manuscript accepted: 30 April 2006

Abstract. A pronounced decline in catch of brown trout (*Salmo trutta* L.) over the last 10–20 years has been reported for many rivers in Switzerland. Impaired health status of the fishes has been suggested as one possible cause of the decline. The present study investigated the health status of juvenile brown trout from four Swiss rivers which experienced reductions of brown trout catches during the last two decades: Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. A gradient approach was applied, studying at each river a headwater (HW), midstream (D1) and downstream site (D2). Fish health was assessed by the following indices: hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity, organ (liver, kidney) histopathology, and gross biometric indices (condition factor, hepatosomatic index). Hepatic EROD activities were generally low without showing significant within- or between-stream differences. Histopathological alterations of the liver displayed a moderate downstream increase in the Emme, LBK and Necker, but not in the Venoge. Between-stream differences of liver pathology were small. Kidney histopathology was not different between upstream and downstream sites, except for Emme and Venoge, where fishes at the downstream sites were infected with the parasite *Tetracapsuloides bryosalmonae,* the cause of the proliferative kidney disease (PKD). The findings from this study point to an association of within-stream gradients in water quality, PKD prevalence, fish health and brown trout biomass, whereas between-stream differences of actual fish health status reflecting the different levels of catch declines observed in the four rivers during the last two decades are not evident.

Key words: Brown trout; EROD; histopathology; kidney; liver; proliferative kidney disease; fish health; biomarker.

Introduction

Over the past few decades several lines of evidence indicated that brown trout (*Salmo trutta*) populations in Switzerland are at risk. On average, catch of brown trout has declined by approximately 40–50% over the last 10–20 years (Burkhardt-Holm et al., 2002). In parallel, brown trout with macroscopic lesions and histopathological alterations have been observed in a number of Swiss rivers, pointing to an impaired health status of the fish (Bernet et al., 2000; Schmidt-Posthaus et al., 2001). Based on such observations, the project "Fischnetz" ("Fish net" – national project on declining fish catch) was initiated which aimed to evaluate possible causes of the fish catch decline in Swiss rivers (Burkhardt-Holm et al., 2002; Fischnetz, 2004; Burkhardt-Holm et al., 2005).

"Fischnetz" formulated several hypotheses on the possible causes of the impairment of brown trout popula-

* Corresponding author phone: +41-31 631 2441;
fax: +41-31 631 2611; e-mail: helmut.segner@itpa.unibe.ch
Published Online First: March 2, 2007

tions in Switzerland, including, among others, poor water quality, altered habitat structure, increased loads of fine sediments, increased water temperature, or altered angler behaviour and fisheries management (see Burkhardt-Holm et al., 2002; 2005). The present study addresses the hypothesis that an impaired health status of brown trout in Swiss rivers contributes to the observed catch decline. Factors such as pathogens, insufficient food resources, or environmental stressors such as high water temperature or toxicant exposure influence fish health, possibly leading to altered fish survival, recruitment, and, ultimately, population growth. Since a retrospective assessment of fish health over the last 10 to 20 years when catches of brown trout were declining was not possible, we investigated the actual health status of brown trout in four Swiss rivers which have experienced different levels of catch reduction during the last 20 years. Further, for each of the four rivers an instream gradient was studied (Downes et al., 2002), i.e. comparing fish health at upstream, midstream and downstream reaches which were characterised by different abundances of brown trout. This within-stream and between-streams comparison provides the option to correlate variations of fish abundance, fish health and possible causative factors such as pathogens or water quality.

Four river basins were selected for health assessment of brown trout: the Emme, the Liechtensteiner Binnenkanal (LBK), the Necker and the Venoge. During the last 20 years, catches of the main resident fish species in these four streams, the brown trout (*Salmo trutta*), declined by 25 to 90%. Since fish health is a multi-parametric endpoint, we used a combination of several, both specific and integrative health indices (cf. Adams et al., 1989; Triebskorn et al., 2001; Broeg et al., 2005): 7-ethoxyresorufin-O-deethylase (EROD) activity was measured to indicate exposure of brown trout to dioxin-like xenobiotics and polycyclic aromatic hydrocarbons (PAH). These contaminants have been involved in several cases of declining fish populations (Fairbrother et al., 1999; Rolland, 2000; Whyte et al., 2000; van der Oost et al., 2003). Organ histopathology was examined since histopathological changes integrate the impact of a variety of stressors including pathogens, toxic compounds or unfavourable nutritional and temperature conditions and for this are valuable markers of environmental stress (Hinton et al., 1992; Segner and Braunbeck, 1988, 1990; Schmidt-Posthaus et al., 2001; Au, 2004). In addition, impaired organ structure and function can directly affect survival, growth and reproduction of the organism (Chavin, 1973; Stebbing, 1985; Segner and Braunbeck, 1988; Teh et al., 1997; Wahli, 2002). Finally, gross indices such as condition factor (CF) and hepatosomatic index (HSI) were determined since they represent integrative indicators of fish condition (Mayer et al., 1992). The study was performed over a two-year-period in order to estimate the year-to-year variability of the investigated parameters.

Materials and methods

Sampling

In each of the four rivers, fish were sampled at three sites, one headwater site (HW) near the river source which was in three of four rivers not impacted by wastewater effluents from sewage treatment plants (STPs), and two more anthropogenically influenced sites downstream of the river: midstream site (D1) and downstream site (D2). In the Emme, Necker and upper Venoge, the sites were either separated by barriers or the distance between the sites was large enough to consider fish migration negligible. In the lower Venoge and LBK, however, some migration may occur. Sampling was done in August 2002 and in August 2003. At each site in each river, 20 juvenile brown trout (total length from 8.4 to 22.4 cm) were collected. Due to the decline of the brown trout populations in the four rivers, a sample size of 20 fish could not be achieved at each site: in the river Emme, at D2 (n = 6), at D1 (n =19) in 2002, and in the following year, at D1 (n = 14) and no fish at all at D2; in the Necker, in 2003 only 16 at D2 and 14 at HW; in LBK, at D2, 15 fish, at D1, 13 fish and at HW19 fish in 2003, no fish at D2 in 2002. In the Venoge, a drought made sampling impossible at the HW site in 2003.

Determination of water quality parameters at the sampling sites was done by cantonal laboratories (Environmental Protection Office St. Gallen, Environmental Protection Office Liechtenstein, Water and Soil Protection Laboratory of Bern, Water and Soil Protection Laboratory of Vaud) which analysed once per month the following parameters: dissolved organic carbon, nitrate, nitrite, ammonium, orthophosphate and total phosphorus. The water temperature was measured by means of data loggers (Minilog) at each site once or twice per hour during the whole year (Schager and Peter, 2004).

Analytical procedures

Fish were caught by electrofishing. After capture, length and weight (total weight, including viscera) of the fishes were measured. Then, the fishes were sacrificed by an overdose of MS 222 (tricaine methane-sulfonate 250 mg/l). A standard necropsy was performed and sex of the fishes was recorded. Liver and kidney were removed and the liver was weighted to determine the hepatosomatic index (HSI). The condition factor CF was calculated as $K = 100 * W/L^3$, where W = body weight (g) and L = length (cm). The HSI was calculated as ratio of liver mass to body mass.

Hepatic EROD activity was measured using the kinetic enzyme assay based on the protocol of Behrens and Segner (2001). Briefly, the cranial part of the liver was shock-frozen in liquid nitrogen before storage at –80 °C until use. After thawing, liver tissue (0.1 g) was homoge-

nized on ice using a Elvehjem potter in 1 ml of homogenisation buffer (50 mM Tris, 2 M saccharose, 2 mM Na_2-EDTA, 150 mM KCl, 0.2 mM dithiothreitol, 0.2 mM phenylmethylsulfonylfluoride; pH 7.6). The homogenate was centrifuged at 10,000 g at 4 °C for 20 min. The supernatant was carefully removed and centrifuged for 1 h at 100,000 g at 4 °C in an ultracentrifuge. The pellet containing the microsomal fraction was resuspended in 200 µl phosphate buffer (80 mM Na_2HPO_4, 20 mM KH_2PO_4, 150 mM KCl; pH 7.6). Directly before the measurement of the EROD activity, the resuspended microsomes were diluted 1:5 with phosphate buffer. EROD activities were measured in a fluorescence plate reader (Multilable Counter 1420-011 VICTOR2). In the wells of opaque microtiter plates, 300 µl phosphate buffer containing 47 µM NADPH and 0.5 µM ethoxyresorufin were mixed with 5 µl sample and the amount of resorufin produced during 300 s at 21 °C was determined at the excitation wavelength of 544 nm and at the emission wavelength of 590 nm. The reaction velocity was calculated from the linear portion of the reaction curve and was transformed into pmol resorufin by means of a resorufin standard curve. The standard curve was established by determining the fluorescence of serial resorufin concentrations (62.5–500 nM). EROD activity was expressed as pmol of resorufin formed per min per mg microsomal protein. Each sample was measured in duplicate. The mean value was used for subsequent calculations. Microsomal protein was determined by Bio-Rad DC protein assay.

For histological and immunohistological examinations, pieces of the kidney and of the caudal part of the liver from all fish were fixed in buffered 4% formalin, embedded in paraffin wax and sectioned according to routine histological protocols. The 5 µm sections were stained with hematoxylin and eosin (HE). Glass slides were code-labelled and were examined "blind".

Histological responses were semi-quantitatively evaluated according to a protocol proposed by Bernet et al. (1999). For each organ investigated, the pathological changes were classified into five reaction patterns: Circulatory disturbances (C), regressive changes (R), progressive changes (P), inflammation (I) and neoplasms (N). For liver and kidney, an index was calculated for each reaction pattern, e.g. for the liver: Circulatory liver index (IL-C), regressive liver index (IL-R), inflammatory liver index (IL-I) and neoplastic liver index (IL-N). The sum of these indices yields the total organ index: IL for the liver and IK for the kidney. These indices are indicative for the extent and intensity of histological alteration in the respective tissue and convert qualitative observations on alterations in tissue morphology into a quantitative value.

In order to separate in the semi-quantitative evaluation fish with pathologically altered organs from those with non-altered organs, we developed a scoring scheme for liver (IL) and kidney (IK). This scheme is based on scorings obtained from the histopathological organ indices of brown trout reared in either tap water ("non-stressed control conditon") or collected from strongly polluted water ("strongly pathological condition"):

- Histological index <10: normal/healthy structure; tissue architecture and histology are well developed and show no impairments or pathological changes
- Histological index 11–20: slight modifications of normal tissue architecture and morphology (e.g. change in cell size) are present.
- Histological index 21–30: moderate modifications of normal tissue architecture and morphology are present.
- Histological index 31–40: pronounced modifications of normal tissue architecture and morphology are present.
- Histological index >40: severe alterations of normal tissue architecture and morphology.

In Emme and Venoge, brown trout populations are infected with the parasite *Tetracapsuloides bryosalmonae* causing the proliferative kidney disease (PKD) (see Wahli et al., 2002). This disease can induce a pronounced inflammatory response of kidney tissue; the diagnosis of PKD-infected fish is therefore essential in the histopathological evaluation of the kidney. Diagnosis of PKD was accomplished on the one hand by histological identification of the PKD-causing parasite *Tetracapsuloides bryosalmonae* in HE-stained sections of the kidney tissue, and on the other hand by immunohistochemical staining of the parasite (see Schubiger, 2003). Differential identification of the kidney parasite, *Sphaerospora sp*., was based on histological and immunohistochemical observations.

Statistics

Biometrical, biochemical and histological data was initially tested for normality using the Kolmogorov-Smirnov test. Most data showed non-normal distribution. The Kruskal-Wallis test, Bonferroni corrected, was used to compare mean values beween sites. Correlations were determined using Sperman's test of correlations. The level of significance of all statistical tests was set at 5%. All statistical analyses were calculated using the NCSS programm version 2001.

Results

Study sites (Tables 1, 2)

The Emme is a mid-sized river of 80 km length extending from the foothills of the Alps to the Swiss midlands. Catch of brown trout has declined by approximately 60%

Table 1. Description of the two downstream (D1, D2) and the upstream (HW) sampling sites at the four rivers, Emme, Necker, Liechtensteiner Binnenkanal (LBK), and Venoge.

Emme	**D2**	**D1**	**HW**
Stream kilometre	59	56.2	9.3
Size (population equivalents) of STP closest to sampling site	40,000	26,000	no STP
Distance of sampling site to STP (km)	4	3	no STP
% effluent in river water at sampling site	2	3	no STP
Brown trout biomass: kg/ha[a] (2002/2003)	0/0.5	0/11.8	42.9/119.6
LBK	**D2**	**D1**	**HW**
Stream kilometre	18	8	4
Size (population equivalents) of STP closest to sampling site	no STP	4,500	no STP
Distance of sampling site to STP (km)	no STP	1	no STP
% effluent in river water at sampling site	no STP	10	no STP
Brown trout biomass: kg/ha[a] (2002/2003)	15.9/13.7	79.3/31.3	91.7/81.3
Necker	**D2**	**D1**	**HW**
Stream kilometre	27	21	6
Size (population equivalents) of STP closest to sampling site	4,500	1,500	no STP
Distance of sampling site to STP (km)	3	1	no STP
% effluent in river water at sampling site	2	1	no STP
Brown trout biomass: kg/ha[a] (2002/2003)	5.7/11.6	34.3/35.6	24.5/47.6
Venoge	**D2**	**D1**	**HW**
Stream kilometre	44 (Venoge)	39 (Venoge)	8 (Veyron)
Size (population equivalents) of STP closest to sampling site	21,250	10,200	1,750
Distance of sampling site to STP (km)	2	1	6
% effluent in river water at sampling site	10.2	15	2
Brown trout biomass: kg/ha[a] (2002/2003)	15.7/6.3	38.9/32.3	311.2/no sampling[b]

[a] Fish abundance was determined by means of electrofishing by Dr. Eva Schager, Swiss Federal Institute for Environmental Science and Technology (Eawag), Kastanienbaum. A more detailed description of the fish population status at the study sites is given in Schager and Peter, 2004.

[b] No fish sampling possible in summer 2003 due to drought

STP: sewage treatment plant.

since 1990 (Scheurer, 2004; Burkhardt-Holm et al., 2006). During the study period (2002–2003), brown trout biomass differed strongly between upstream and downstream sites (Table 1, Schager and Peter, 2004). The river

Table 2. Water quality parameters of the downstream (D1, D2) and upstream (HW) sampling sites at the four rivers, Emme, Necker, Liechtensteiner Binnenkanal (LBK) and Venoge.

Emme	**D2**	**D1**	**HW**
DOC [mg C/l]	4.22 *	2.86	3.40
NO_3-N [mg N/l]	3.76	2.51	0.52
NH_4-N [mg N/l]	0.31	0.09	< 0.01
Ptot [mg P/l]	0.11	0.05	0.02
Temperature range July and August 2002 (°C)	9.2–20.2	11.1–18.4	11.2–23.1
Yearly mean (°C) 2002	10.3	9.1	6.6
Temperature range July and August 2003 (°C)	13.4–25.2	12.5–21.7	10.5–22.8
Yearly mean (°C) 2003	10.4	9.6	7.0
LBK	**D2**	**D1**	**HW**
DOC [mg C/l]	1.19	1.30	0.80
NO_3-N [mg N/l]	0.89	1.50	1.00
NH_4-N [mg N/l]	0.02	0.45*	0.08
P_{tot} [mg P/l]	0.01	0.03	0.02
Temperature range July and August 2002	9.3–15.4	10.2–14.6	10.4–13.2
Yearly mean (°C) 2002	8.7	9.8	8.5
Temperature range July and August 2003	9.5–15.5	10.6–18	11.1–14.9
Yearly mean (°C) 2003	8.9	10.2	8.9
Necker	**D2**	**D1**	**HW**
DOC [mg C/l]	1.90	1.92	1.30
NO_3-N [mg N/l]	1.27	1.24	1.00
NH_4-N [mg N/l]	0.03	0.03	<0.01
P_{tot} [mg P/l]	0.03	0.06	0.02
Temperature range July and August 2002	n.d.	n.d.	9.0–15.8
Yearly mean (°C) 2002	7.6	7.3	6.6
Temperature range July and August 2003	11.2–25.6	10.2–24.4	9.2–16.8
Yearly mean (°C) 2003	9.0	8.9	6.8
Venoge	**D2**	**D1**	**HW**
DOC [mg C/l]	3.46	2.88	3.62
NO_3-N [mg N/l]	3.95	3.71	2.39
NH_4-N [mg N/l]	0.16	0.09	0.02
P_{tot} [mg P/l]	0.09	0.08	0.04
Temperature range July and August 2002	12.0–20.2	12.4–18.6	10.4–14.2
Yearly mean (°C) 2002	10.1	9.7	8.1
Temperature range July and August 2003	16.8–24.4	15.9–24.0	12.1–16.8
Yearly mean (°C) 2003	11.8	11.6	8.8

For dissolved organic carbon (DOC), nitrate, ammonia, and total phosphorus, the 80 percentiles are given for monthly measured samples in 2002.

* = Concentrations that exceeded the quality aims for running water according to the Swiss water pollution control decrete (GSchV, 1998): DOC [mg C/l] < 4, NH_4-N [mg N/l] < 0.4.

For the temperature range indicated for July and August, minimum and maximum values during this period are given.

n.d.= not determined.

Chemical analyses were provided by cantonal laboratories (Environmental Protection Office St. Gallen, Environmental Protection Office Lichtenstein, Water and Soil Protection Laboratory Bern, Water and Soil Protection Laboratory Vaud).

is considerably influenced by spring snowmelt and seasonal flow fluctuation. The mean flow rate varies from 1.1 m^3/s at the headwater (HW) site to 14.1 m^3/s the most downstream (D2) site. The construction of small dams and weirs has resulted in fragmentation, isolation of tributaries, and poor riparian conditions. Additionally, high water extraction takes place, primarily for agriculture. Natural trout habitats are found mostly in the upper reach. Total catchment area of the Emme is approximately 963 km^2 and is characterized mainly by forest (40% of the catchment area), and farmland (35%, mostly downstream). Buildings and streets take 5% of the area (Strehler, 2003). The stream receives effluents from three larger STPs (Table 1), while a number of smaller STPs discharge into the river's tributaries. The concentrations of the analysed nutrients including NO_3-N, NH_4-N, and P_{tot} show a pronounced downstream increase, while for DOC a consistent trend was not evident (Table 2). In the lower reach of the Emme, several pesticides including atrazine, diuron and diazinon are present, with atrazine reaching maximum concentrations close to the predicted no-effect concentration (PNEC) of 700 ng/L (Götz et al., 2003; Burkhardt-Holm et al., 2006). Water temperature in the Emme during summer can go up to 20 °C and higher (Table 2), thus exceeding the physiological optimum of brown trout.

The Liechtensteiner Binnenkanal (LBK) is a 29-km-long channel constructed in the 1930s for flood protection and land conversion. Catch of brown trout in the LBK has declined by approximately 90% since 1980 (Scheurer, 2004; Burkhardt-Holm et al., 2006). During the study period (2002–2003), brown trout biomass differed strongly between the upstream and the downstream site (Table 1, Schager and Peter, 2004). Importantly, at both downstream sites (D1 and D2), brown trout lives in competition to rainbow trout (Schager and Peter, 2004). The flow is rather constant, ranging from a mean flow rate of 0.2 m^3/s at the HW site to 1.7 m^3/s at the D2 site. The only prominent barrier, at the mouth of the channel, was removed in the year 2000. Restrictions of natural habitats are mainly due to longitudinal constructions leading to low variability in width and a regulated flow. This situation has led to high levels of stream bed fines and sediment clogging. The catchment area of 138 km^2 is dominated by forest (50%), with only 18% farmland. The area of buildings and streets occupies 10% (Strehler, 2003). There is one STP discharging into the LBK which is located close to D1 (Table 1). Accordingly, the LBK showed highest nutrient values and temperatures at D1 (Table 2), with NH_4-N exceeding the Swiss quality aim for running waters (GSchV, 1998). Further, elevated levels of polybrominated diphenyl ethers were found in trout liver sampled at D1 (Hartmann et al., 2006). Water temperatures of the LBK usually do not exceed 15 °C, even during summer (Table 2).

The Necker is a pre-alpine river in Northeast Switzerland, with a length of 31 km. Catch of brown trout in the Necker has declined by more than half since 1980 (Scheurer, 2004; Burkhardt-Holm et al., 2006). During the study period (2002-2003), size of brown trout populations decreased in the downstream direction, particularly between D1 and D2 (Table 1, Schager and Peter, 2004). The mean flow rate of the Necker is 0.3 m^3/s at the HW site and 0.6 m^3/s at the D2 site. River morphology is only mildly disturbed, providing varied habitats for all life stages of brown trout. The catchment area is 123 km^2, with 38% covered by forest and 35% used for farmland. Buildings and streets make up 4% of the area (Strehler, 2003). The Necker receives effluents from three STPs. Most chemical values as well as temperature showed a downstream increase (Table 2). Pesticide concentrations in the Necker are low (Burkhardt-Holm et al., 2006). Summer water temperatures can go beyond 15 °C, at least at the two lower study sites, D1 and D2 (Table 2).

The Venoge represents a mid-sized river in the west midlands of Switzerland and flows into Lake Geneva. Catch of brown trout in the Venoge has declined by approximately 25% during the last 20 years (Scheurer, 2004; Burkhardt-Holm et al., 2006). In the Venoge, like in the other rivers, an upstream-downstream gradient in brown trout biomass was present (Table 1, Schager and Peter, 2004). The mean flow rate of the Venoge is 0.2 m^3/s at the HW site and 2.5 m^3/s at the D2 site. The Venoge provides many natural or only mildly disturbed habitats, however, several weirs impair fish migration. While the two downstream sampling sites (D1, D2) are located at the Venoge, the headwater site is situated at a tributary stream, the creek Le Veyron. Le Veyron is occasionally desiccated (Table 1). Venoge and Le Veyron together have a catchment area of 231 km^2 and a length of 70 km. Land use in the catchment area of Le Veyron includes 56% forest and 40% intensive agriculture, while the drainage area of the Venoge is dominated by agriculture (57%). Buildings and streets take 8.5% of the area (Strehler, 2003). The stream is influenced by eighteen smaller STPs . Chemical values show a downstream increase, except for DOC (Table 2). The Venoge contains several pesticides and, similarly to the Emme, in the lower reach of the Venoge, atrazine concentrations can reach levels higher than the PNEC of 700 ng/L (Götz et al., 2003, Burkhardt-Holm et al., 2006). Summer water temperatures go beyond 15 °C (Table 2).

Gross body indices of brown trout

The condition factors (CF) of the fish collected in 2002 and 2003 at the 12 sample sites in the four streams are shown in Figures 1a and 1b. While in Emme and LBK, CF values tended to increase (approximately 3–7%) in the downstream direction, no clear-cut trend was evident in Venoge and Necker. When comparing CF values be-

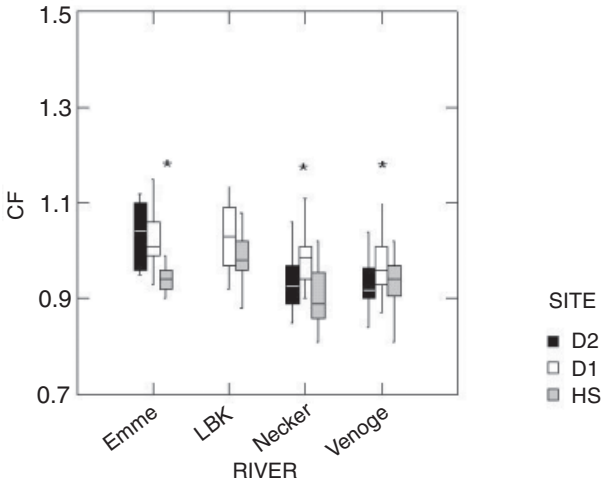

Figure 1a. Condition factor of juvenile brown trout sampled in 2002 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1 and 2.
n-number of sampled fish ranged from 6 to 28. Sampling was done in July and August 2002. No samples were available for D2 of the LBK.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).

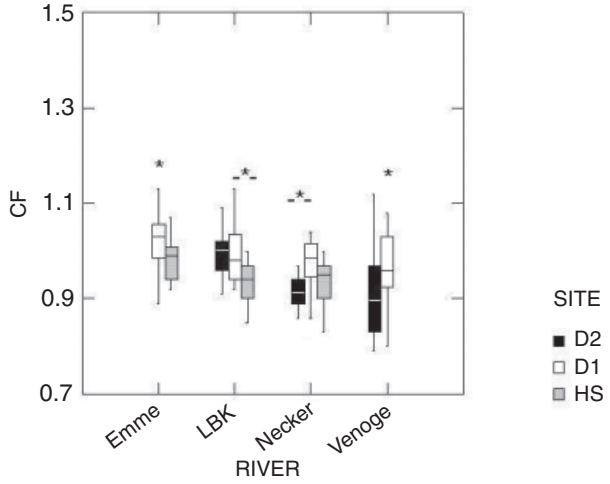

Figure 1b. Condition factor of juvenile brown trout sampled in 2003 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2.
n-number of sampled fish ranged from 13 to 20. Sampling was done in July to August 2003. No samples were available for D2 of Emme and HW of Venoge.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).

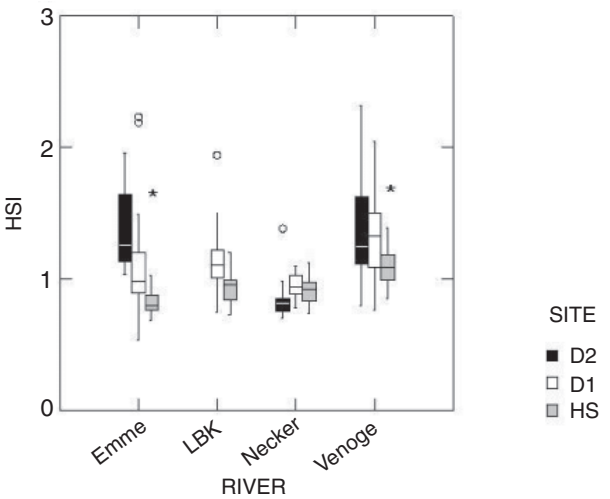

Figure 2a. Hepatosomatic index (HSI) of juvenile brown trout sampled in 2002 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2.
n-number of sampled fish ranged from 6 to 28. Sampling was done in July and August 2002. No samples were available for D2 of LBK.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$), °: outlier.

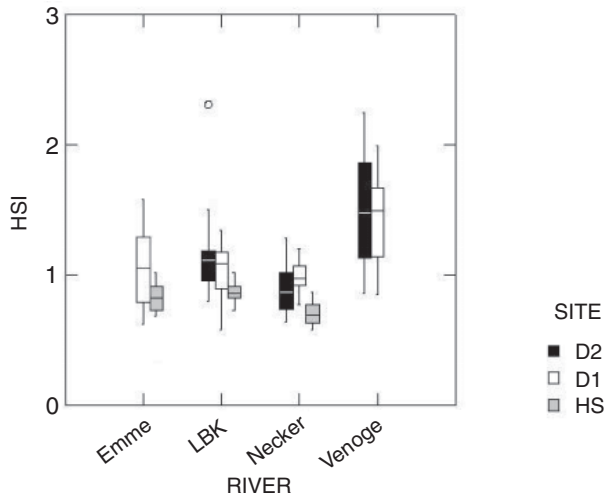

Figure 2b. Hepatosomatic index (HSI) of juvenile brown trout sampled in 2003 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2.
n-number of sampled fish ranged from 13 to 20. Sampling was done in July and August 2003. No samples were available for D2 of Emme and HW of Venoge.
°: outlier.

tween the corresponding sampling sites of the four rivers, there existed no significant between-stream differences, with the exception that in 2002, CF values of fish at HW of Necker were significantly lower than CF values at HW of LBK (Kruskal-Wallis test, Bonferroni adjusted, $n_{Necker} = 20$, $n_{LBK} = 25$; $p < 0.05$). Concerning between-year variation of CF values, for none of the 12 sampling sites significant differences could be observed. CF values of male and female juvenile trout were not significantly different, when calculated for all sampling sites, however,

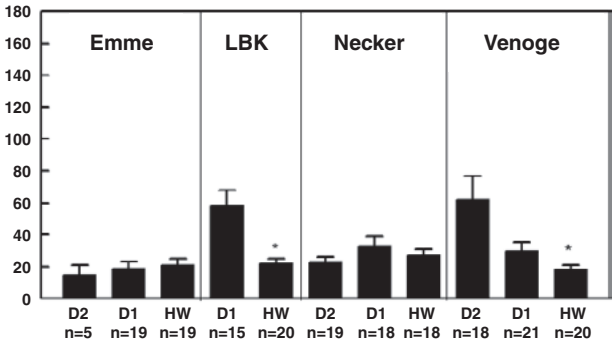

Figure 3a. Mean hepatic EROD activities ± standard error of brown trout sampled in 2002 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge.
N = number of analyzed fish.
For details of the sampling sites: see text and tables 1, 2. Sampling was done in July and August 2002. No samples were available for D2 of LBK.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).

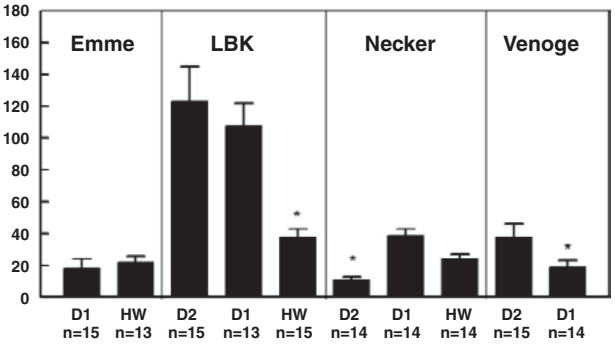

Figure 3b. Mean hepatic EROD activities ± standard error of brown trout sampled in 2003 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge.
N = number of analyzed fish.
For details of the sampling sites: see text and tables 1, 2. Sampling was done in July and August 2003. No samples were available for D2 of Emme and HW of Venoge.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).

significant gender differences were observed at individual sites, namely for D1 at LBK in 2003 (mean: males: 1.22; females 0.96, $p = 0.016$) and for D1 at Necker in 2003 (mean: males 1.01; females 0.96, $p = 0.024$).

Data on the hepatosomatic index (HSI) of the fish collected at the 12 sample sites in the four streams is shown in Figures 2a and 2b. Generally, in all four rivers a trend for increasing HSI values in the downstream direction is indicated. This increase ranged between 2 and 24%, and was significant for Emme and Venoge in 2002. Between-stream differences of HSI values were in no case significant.

EROD activities

In 2002, the highest values of hepatic EROD activity were observed at the site D1 of LBK and at the site D2 of Venoge (Fig. 3a). The lowest mean value of EROD activity (11 pmol/mg protein/min) was found in fish at the site D2 of the Emme. In LBK and Venoge, fish from the HW sites showed significantly lower EROD values than fish at the more downstream sites, but for Emme and Necker no significant between-site differences existed. In 2003, particularly fishes from the LBK tended to show higher mean EROD levels (107 to 128 pmol/mg protein/min) than in 2002 (Fig. 3b), however, the differences were not significant. The only sampling site for which a significant between-year difference was noted, was D2 at the Venoge (Kruskal-Wallis test, Bonferroni adjusted, $n_{2002} = 16$, $n_{2003} = 15$; $p < 0.05$). As in 2002, also in 2003 EROD values of fishes from the HW site of the LBK were significantly lower than in fish from D1 and D2 of the LBK (in the Venoge, due to draught, sampling of the HW site was not possible in 2003). A general trend for increasing EROD values in the downstream direction of the four rivers (i.e. in parallel to the increasing load of wastewater and chemicals) was not evident. Only in the LBK and in the Venoge in 2002, downstream fish showed increased hepatic EROD activities, while in Emme and Necker, at least in 2003 trout from downstream exhibited significantly lower EROD activities than fish from the upstream site (Fig. 3b). Hepatic EROD values of male and female juvenile trout did not differ significantly.

Liver histopathology

Liver lesions were found at all sites but the prevalence and the severity differed between the sites. Using the histomatrix approach of Bernet et al. (1999) (see Material and methods), alterations of hepatic histology were summarized into one numerical value, the total liver index (IL). This index is composed of five types of liver lesions: regressive, progressive, inflammatory, circulatory and neoplastic changes. Circulatory disturbances and neoplasms were not detected in any of the 415 livers examined. Regressive alterations (quantified as regressive liver index IL-R) contained mainly a change of tissue structure and architecture, such as distended sinusoids, separation of liver cells, hepatocellular and nuclear pleomorphism, changes in cellular cytoplasm, e.g., strongly eosinophilic or granulated cytoplasm, single cell necrosis or necrotic foci. Progressive liver alterations (quantified as progressive liver index IL-P) consisted mainly of an increased number of mitotic liver parenchymal cells and, occasionally, pericholangiar fibrosis. The inflammatory index (IL-I) contained changes such as activation of the reticulo-endothelial system, and/or liver infiltration with lymphocytes and macrophages, either around

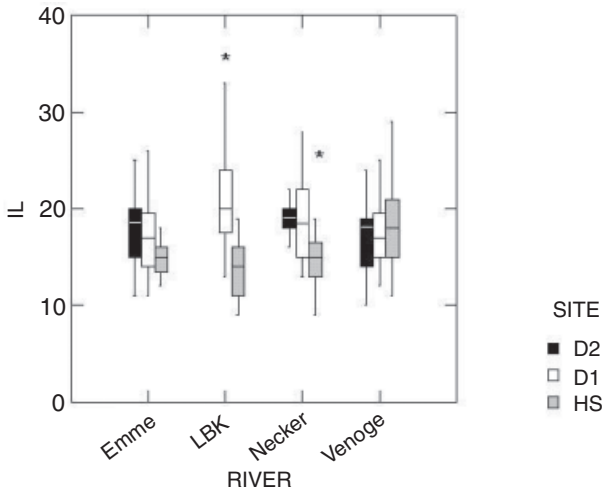

Figure 4a. Histological liver index (IL) of brown trout sampled in 2002 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2. n-number of sampled fish ranged from 6 to 28. Sampling was done in July and August 2002. No samples were available for D2 of LBK.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).

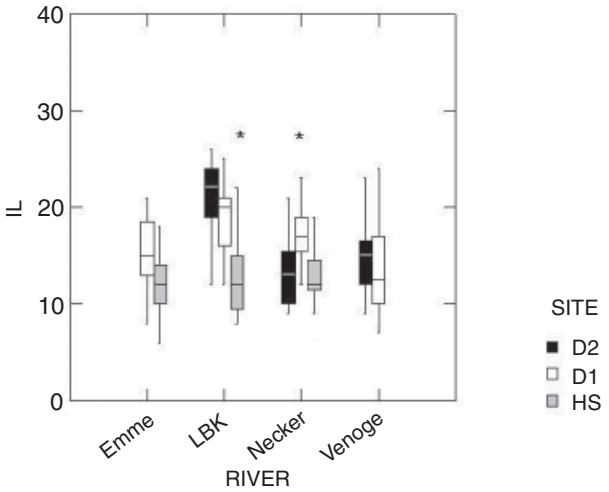

Figure 4b. Histological liver index (IL) of brown trout sampled in 2003 at the downstream (D1, D2) and upstream (HW) sites of the four rivers, Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2. n-number of sampled fish ranged from 13 to 20. Sampling was done in July and August 2003. No samples were available for D2 of LBK.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).

bile ducts and ductuli, or randomly distributed in the parenchyma.

IL values of individual fish differed between 5 and 33 among the 415 livers examined in this study. If classifying livers with an IL value of <10 to represent undisturbed liver structure, 6% of the examined livers were in this range. Among the remaining fish, 79% showed livers with slight modifications, i.e. an IL between 11–20, 14% of the livers were moderately altered (IL between 21–30) and 1% of the livers showed severe alterations, with IL values of 31 and more. The latter livers regularly showed necrotic foci. Interannual differences of the IL were not significant, with the exception of the Necker where fish of the D2 showed significantly lower IL values in 2003 than in 2002 (Kruskal-Wallis test, Bonferroni adjusted, $n_{2002} = 28$, $n_{2003} = 16$; $p < 0.05$).

In the study period 2002, mean IL values of trout from the rivers Emme, LBK and Necker showed a downstream increase (Fig. 4a). The downstream change was significant for LBK and Necker, but not for Emme. In the Venoge, a downstream increase was not evident. Concerning IL-R, IL-P, and IL-I, HW fish tended to have lower values, but the differences were not significant, except for the inflammatory index IL-I, which showed a significant downstream increase in trout sampled in the Emme (Table 3). Comparing between-stream differences, IL values of fish from the HW of Venoge were significantly higher than of fish from the HW sites of Emme, LBK and Necker (Kruskal-Wallis test, Bonferroni adjusted, $n_{Venoge} = 26$, $n_{Emme} = 23$, $n_{Necker} = 20$, $n_{LBK} = 25$; $p > 0.05$). In 2003, similar trends of IL values as described for 2002 were observed, with the exception of the Necker, where fish from the D1 site had significantly higher IL values than fish from HW and D2 (Fig. 4b).

Kidney histopathology

The alterations of kidney histology were summarized into one numerical value, the total kidney index (IK). This index is composed of five types of kidney lesions: regressive, progressive, inflammatory, circulatory and neoplastic. As already observed for the liver, circulatory disturbances and neoplastic changes were not detected in any of the 408 kidneys examined. Regressive alterations (quantified as regressive kidney index IK-R) consisted mainly of single pyknotic nuclei, single necrotic tubular epithelial cells, deposits of desquamating tubular cells, hyalinous casts in the lumina of the tubuli, changes in cytoplasmic appearance of epithelial cells such as eosinophilic granulated cytoplasm, deposits in the Bowman's capsule and altered architecture of the glomeruli. In fish infected with the causative agent of the proliferative kidney disease (PKD), *Tetracapsuloides bryosalmonae,* regressive changes included multifocal necrosis of interstitial tissue and tubuli. Progressive alterations (quantified as progressive kidney index IK-P) consisted mainly of an increased frequency of proliferating tubuli and a proliferation of interstitial tissue as being associated with an infection by *Tetracapsuloides bryosalmonae*. The inflammatory kidney index (IK-I) was characterized mainly by granulomatous infiltration in PKD-infected fish. Pres-

Table 3. Histological indexes (mean ± standard error) of fishes sampled at downstream (D2), midstream (D1) and upstream (HW) sites of the four rivers Emme, Necker, Liechtensteiner Binnenkanal (LBK) and Venoge in the years 2002 and 2003.

Emme	**D2 2002**	**D2 2003**	**D1 2002**	**D1 2003**	**HW 2002**	**HW 2003**
IL	18.00 ± 1.63 (6)	(0)	17.68 ± 0.91 (19)	15.20 ± 0.88 (20)	14.00 ± 0.83 (23)	12.07 ± 1.05 (14)
IK	22.33 ± 3.31 (6) a	(0)	19.06 ± 1.91 (18) b	21.50 ± 1.61 (20) A	10.78 ± 1.69 (23) a, b	8.64 ± 1.92 (14) A
IL R	6.66 ± 1.13 (6)	(0)	8.00 ± 0.64 (19)	4.40 ± 0.50 (20)	6.22 ± 0.58 (23)	4.43 ± 0.60 (14)
IL P	2.33 ± 0.57 (6)	(0)	2.00 ± 0.32 (19)	1.2 ± 0.32 (20)	1.91 ± 0.29 (23)	2.00 ± 0.39 (14)
IL I	9.00 ± 0.86 (6) a	(0)	7.68 ± 0.48 (19) b	9.60 ± 0.47 (20) A	6.74 ± 0.44 (23) a, b	5.64 ± 0.57 (14) A
IK R	13.33 ± 1.53 (6)	(0)	12.72 ± .088 (6)	12.50 ± 0.67 (20) A	10.35 ± 0.78 (23)	8.36 ± 0.80 (14) A
IK P	5.67 ± 1.44 (6) a	(0)	4.11 ± 0.83 (18) b	6.00 ± 0.79 (20) A	0.43 ± 0.74 (23) (23) a b	0.28 ± 0.94 (14) A
IK I	3.33 ± 0.90 (6) a	(0)	2.22 ± 0.52 (18) b	3.00 ± 0.44 (20) A	0.00 (23) a, b	0.00 (14) A
LBK	**D2 2002**	**D2 2003**	**D1 2002**	**D1 2003**	**HW 2002**	**HW 2003**
IL	(0)	21.13 ± 1.00 (15) A	20.96 ± 0.80 (27) a	18.69 ± 1.08 (13) B	13.84 ± 0.83 (25) a	12.37 ± 0.89 (19) A, B
IK	(0)	10.29 ± 0.52 (14)	10.42 ± 0.41 (26)	11.23 ± 0.53 (13)	11.0 ± 0.42 (25)	9.58 ± 0.47 (17)
IL R	(0)	7.93 ± 0.63 (15) A	9.60 ± 0.58 (27) a	6.46 ± 0.68 (13)	6.92 ± 0.60 (25) a	4.89 ± 0.56 (19) A
IL P	(0)	4.67 ± 0.45 (15) A	2.67 ± 0.33 (27) a	3.23 ± 0.49 (13) B	1.44 ± 0.34 (25) a	1.47 ± 0.40 (19) A, B
IL I	(0)	8.53 ± 0.47 (15) A	8.48 ± 0.35 (27) a	9.00 ± 0.51 (13) B	5.48 ± 0.37 (25) a	6.00 ± 0.42 (19) A, B
IK R	(0)	8.71 ± 0.46 (14)	9.42 ± 0.39 (26) a	9.84 ± 0.48 (13)	10.84 ± 0.40 (25) a	9.23 ± 0.42 (17)
IK P	(0)	1.29 ± 0.28 (14)	0.92 ± 0.16 (26) a	0.92 ± 0.31 (13)	0.16 ± 0.16 (25) a	0.35 ± 0.25 (17)
IK I	(0)	0.29 ± 0.17 (14)	7.70 ± 5.50 (26)	0.46 ± 0.17 (13)	0.00 (25)	0.00 (17)
Necker	**D2 2002**	**D2 2003**	**D1 2002**	**D1 2003**	**HW 2002**	**HW 2003**
IL	19.10 ±0.61 (28) a	13.50 ± 0.82 (16) A	18.95 ±0.69 (22) b	17.25 ± 0.74 (20) A, B	14.60 ± 0.72 (20) a, b	13.10 ± 0.76 (19) B
IK	10.18 ± 0.42 (28)	9.63 ± 0.59 (16) A	9.60 ± 0.47 (22)	11.65 ± 0.52 (20) A, B	8.60 ± 0.49 (19)	7.74 ± 0.54 (19) B
IL R	9.90 ± 0.40 (28) a	4.31 ± 0.43 (16)	9.77 ± 0.45 (22) b	5.00 ± 0.39 (20)	7.35 ± 0.47 (20) a, b	4.11 ± 0.40 (19)
IL P	1.71 ± 0.32 (28) a	1.88 ± 0.45 (16) A	2.90 ± 0.36 (22) a, b	3.8 ± 0.40 (20) A, B	1.50 ± 0.37 (20) b	0.53 ± 0.41 (19) B
IL I	7.5 ± 0.26 (28) a, b	7.31 ± 0.40 (16) A, B	6.27 ± 0.30 (22) a	8.45 ± 0.36 (20) A	5.75 ± 0.31 (20) b	8.47 ± 0.37 (19) B
IK R	9.39 ± 0.40 (28)	9.38 ± 0.51 (16) A	9.05 ± 0.46 (22)	10.35 ± 0.46 (20) B	8.40 ± 0.48 (19)	7.53 ± 0.47 (19) A, B
IK P	0.79 ± 0.16 (28) a	0.00 (16) A	0.55 ± 0.18 (22)	1.00 ± 0.15 (20) A, B	0.10 ± 0.19 (19) a	0.11 ± 0.16 (19) B
IK I	0.00 (28)	0.25 ± 0.19 (16)	0.00 (22)	0.3 ± 0.17 (20)	0.00 (19)	0.11 ± 0.17 (19)
Venoge	**D2 2002**	**D2 2003**	**D1 2002**	**D1 2003**	**HW 2002**	**HW 2003**
IL	16.91 ± 0.80 (23)	14.55 ± 0.88 (20)	17.33 ± 0.79 (20)	13.56 ± 0.99 (16)	18.42 ± 0.76 (26)	(0)
IK	16.87 ± 1.44 (23) a	11.74 ± 0.72 (19)	12.12 ± 1.41 (24)	9.88 ± 0.78 (16)	9.87 ± 1.36 (26) a	(0)
IL R	8.17 ± 0.56 (23)	5.5 ± 0.65 (20)	7.58 ± 0.55 (24)	4.56 ± 0.73 (16)	8.23 ± 0.53 (26)	(0)
IL P	1.30 ± 0.34 (23) a	1.30 ± 0.40 (20)	2.24 ± 0.34 (24)	1.00 ± 0.44 (16)	3.46 ± 0.32 (26) a	(0)

Table 3. Continued

Venoge	D2 2002	D2 2003	D1 2002	D1 2003	HW 2002	HW 2003
IL I	7.43 ± 0.45 (23)	7.75 ± 0.58 (20)	7.42 ± 0.44 (24)	8.00 ± 0.65 (16)	6.70 ± 0.42 (26)	(0)
IK R	12.43 ± 0.61 (23) a	9.95 ± 0.4 (19)	10.63 ± 0.60 (24)	9.75 ± 0.46 (16)	9.70 ± 0.57 (26) a (16)	(0)
IK P	2.96 ± 0.56 (23) a	1.05 ± 0.24 (19)	1.17 ± 0.55 (24)	0.00 (16)	7.69 ± 0.53 (26) a	(0)
IK I	1.48 ± 0.39 (23) a	0.74 ± 0.35 (19)	0.33 ± 0.39 (24)	0.13 ± 0.38 (16)	0.07 ± 0.37 (26) a	(0)

IL = total liver index, IK = total kidney index, IL R = the regressive liver index, IL P = the progressive liver index, IL R = the inflammatory liver index, IK R = the regressive kidney index, IK P = the progressive kidney index, IK I = the inflammatory kidney index. Number of sampled fish is shown in parentheses. a, b = significant differences of indexes between sampling sites in summer 2002; A, B = significant differences of indexes between sampling sites in summer 2003 (Kruskal-Wallis Test, Bonferroni adjusted; $p < 0.05$).

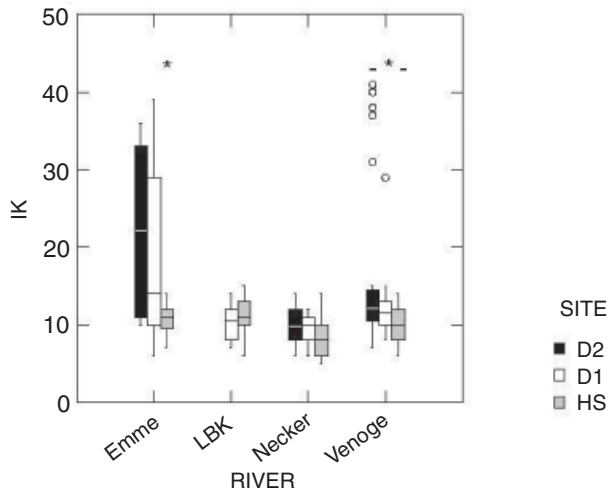

Figure 5a. Histological kidney index (IK) of fishes sampled in 2002 at the downstream (D1, D2) and upstream (HW) sites of the four rivers Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2.
n-number of sampled fish ranged from 6 to 28. Sampling was done in July and August 2002. No samples were available for D2 of LBK.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).
°: outlier.

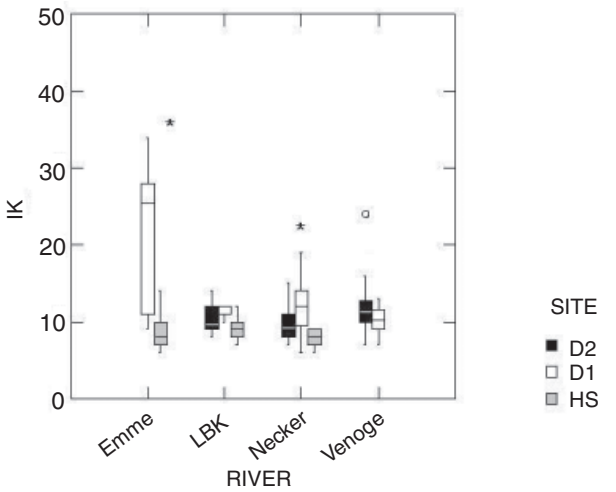

Figure 5b. Histological kidney index (IK) of fishes sampled in 2003 at the downstream (D1, D2) and upstream (HW) sites of the four rivers Emme, Liechtensteiner Binnenkanal (LBK), Necker and Venoge. For details of the sampling sites: see text and tables 1, 2.
n-number of sampled fish ranged from 13 to 20. Sampling was done in July and August 2003. No samples were available for D2 of Emme and HW of Venoge.
*: significant differences between sampling sites per river (Kruskal-Wallis-Test, Bonferroni adjusted; $p < 0.05$).
°: outlier.

ence of another myxozoan parasite, *Sphaerospora* sp., in the kidney tubules was not associated with pathological alterations.

IK values of individual fish differed between 5 and 41. 52% of the kidneys showed an IK value in the range of 5–10, what is considered to represent normal, undamaged tissue, 40% of the IK values were in the range of 11–20 showing slight alterations, 4% were moderately altered (IK values between 21–30), 3% showed pronounced alterations corresponding to IK values between 31–40 and 1% of the fish examined possessed severely altered kidney structures, with IK values higher than 40. Comparing the two years, 2002 and 2003, there was no significant interannual difference of the IK values for all sampling sites.

Infection with the PKD-causing parasite, *Tetracapsuloides bryosalmonae,* was a major factor influencing kidney histopathological scores. PKD-positive fish yielded IK values of 26 at minimum. PKD-diseased trout were found in Emme and Venoge at the two downstream sites D1 and D2, but not in LBK and Necker. In the Emme, PKD prevalence was between 29 and 60%, both in 2002 and 2003. On the contrary, in the Venoge, infected fish were observed only in 2002, and then the prevalence was between 4 and 21% (Table 4). Mean IK values at the PKD-positive sites D1 and D2 of Emme and Venoge were elevated (Figs. 5a, b), as a result of a significant increase of mean values of the progressive index, IK- P, and of the inflammatory index, IK-I, in the downstream reaches of the PKD-positive rivers (Table 3). The increase of mean

Table 4. Prevalence (%) of fish with kidney parasites at the downstream (D1, D2) and upstream (HW) sampling sites of the four rivers, Emme, Necker, Liechtensteiner Binnenkanal (LBK) and Venoge. Number of sampled fish is shown in parentheses.

	D2 2002	D2 2003	D1 2002	D1 2003	HW 2002	HW 2003
Emme						
T. bryosalmonae	50 (6)	0 (0)	38.89 (18)	60 (20)	0 (23)	0 (14)
Sphaerospora sp.	66.67 (6)	0 (0)	72.22 (18)	70 (20)	47.83 (23)	42.86 (14)
LBK						
T. bryosalmonae	0 (0)	0 (14)	0 (26)	0 (13)	0 (25)	0 (17)
Sphaerospora sp.	0 (0)	64.29 (14)	69.23 (26)	69.23 (13)	28 (25)	58.82 (17)
Necker						
T. bryosalmonae	0 (28)	0 (16)	0 (22)	0 (20)	0 (19)	0 (19)
Sphaerospora sp.	53.57 (28)	25 (16)	90.90 (22)	25 (20)	25 (19)	15.79 (19)
Venoge						
T. bryosalmonae	21.74 (23)	0 (19)	4.17 (24)	0 (16)	0 (26)	0 (0)
Sphaerospora sp.	65.22 (23)	89.47 (19)	83.33 (24)	93.75 (16)	73.08 (26)	0 (0)

T. bryosalmonae = Tetracapsuloides bryosalmonae.

values of the kidney indices was much stronger at PKD-positive sites of the Emme than of the Venoge (Fig. 5a, Table 3), but also prevalences were higher at the Emme than at the Venoge (Table 4). Comparing IK values of corresponding sampling sites across rivers revealed that fish from PKD-positive sites showed significantly higher mean IK values than fish from the corresponding sites in the PKD-negative rivers. No PKD infections were found in kidneys of fish from Necker or LBK (Table 4). In these two rivers, no consistent gradient of mean IK values along the river was obvious (Figs. 5a, b).

Discussion

This study aimed a) to assess by a combination of several indices (EROD activity, organ histopathology, biometrical indices) the health status of brown trout from four different streams and from three different sites within each stream, and b) to relate fish health to environmental quality as well as brown trout abundance in the streams. The four river basins investigated – Emme, LBK, Necker, Venoge – differ in their abiotic parameters, including the level of anthropogenic impact and habitat quality. Emme and Venoge show elevated chemical pollution and high summer water temperatures, but differ in habitat quality, with poor habitat quality in the Emme and fairly good habitat quality in the Venoge (Schager and Peter, 2004). In contrast to Emme and Venoge, Necker and LBK have low chemical pollution and lower summer water temperatures. This situation combines in the Necker with an overall good habitat quality, but with poor habitat quality in the LBK. For Emme, Necker and Venoge, water quality impairs in the downstream direction that is from the HW sites over D1 to D2, while in the LBK, poorest water quality is observed at the middle sampling site, D1. Assuming that water quality may directly influence fish health, as it has been shown in numerous studies (e.g., Steyermark et al., 1999; Bernet et al., 2000; Mondon et al., 2001; Stentiford et al., 2003; Broeg et al., 2005), we expected a decrease of fish health status in the downstream direction of the rivers, and a lower health status of brown trout from Emme and Venoge compared to that of brown trout from Necker and LBK. This expectation, however, was only partly met.

Hepatic EROD activity has commonly been employed as a biomarker for exposure to substances binding to the arylhydrocarbon receptor (AhR) such as polynuclear aromatic hydrocarbons (PAHs), and persistent polyhalogenated aromatic hydrocarbons (PHAHs) such as dioxins, furans and polychlorinated biphenyls (Altenburger et al., 2003; Whyte et al.; 2000; van der Oost et al., 2003). Exposure to PAHs and PHAHs can have adverse consequences on fish health and population growth: these xenobiotics are known to adversely affect early life stage survival, development and reproduction of fish (Fairbrother et al., 1999; Rolland, 2000; Altenburger et al., 2003). At field sites with elevated PAH and/or PHAH contamination, such as the Puget Sound or the Great Lakes in Northern America, increased prevalence of toxicopathic lesions and neoplasms (Myers et al., 1998; Stehr et al.; 2003) as well as population declines have been reported (Monosson, 1997). In the present study, the findings of the EROD measurements point to a generally low or even non-existing exposure of brown trout from the four rivers to AhR ligands. According to the available literature, hepatic EROD activities of non-exposed (control) brown trout are in the range of 30 to 50 pmol/mg/min (Whyte et al., 2000; Behrens and Segner, 2005). For the present study we therefore take EROD values of less than 50 pmol/mg/min to represent non-contaminated conditions, while EROD values higher than 50 pmol/mg/

min may indicate exposure of brown trout to inducing chemicals. Among the sites investigated in this study, only at three sites EROD activities of 50 pmol/mg/min and higher were found: the two downstream sites, D1 and D2, of the LBK, and, in 2002, also the most downstream site, D2, of the Venoge. Brown trout from sites D1 and D2 of the LBK, which are downstream to an STP effluent, possessed elevated liver burdens of polybrominated diphenyl ethers (Hartmann et al., 2006) what may contribute to the elevated EROD activities at these sites. Interestingly, brown trout from the D1 and D2 sites of the LBK not only showed increased hepatic EROD levels but also increased prevalence of hepatic morphological changes, particularly mitoses, nuclear alterations and single cell necrosis. Overall, however, hepatic EROD activities of brown trout did not discriminate among the study sites, neither between the four streams, nor between the sites within a stream. This finding indicates that exposure of brown trout to CYP1A-activating chemicals was generally low (with few exceptions – see above), and therefore, probably does not contribute to site- or stream-specific differences of brown trout health or abundance.

Histopathology has been used as a tool to assess health status of wild fish in a number of field studies (Teh et al., 1997; Schwaiger, 2001; Handy et. al., 2002; Schmalz et al., 2002; Stentiford et al., 2003). The advantage of histopathology as a biomarker lies in its intermediate location in the hierarchy of biological organization; therefore, it is able to integrate the effects of both abiotic factors such as chemicals or temperature, and of biotic factors such as pathogens on organ function and fish health (Adams et al., 1989; Teh et al., 1997; Segner and Braunbeck, 1998; Handy et al., 2002). At the same time, the integrative nature of the histopathological alterations implicates that they often cannot be assigned to a specific causative factor, e.g. to a particular toxicant (Meyers and Hendricks, 1985; Hinton et al., 2001; Schmidt-Posthaus et al., 2001). To properly interpret results from histopathological evaluations, it is important to realize that a healthy control condition is not characterized by the complete absence of any histopathological traits, but may display moderate alterations such as minor structural disorders or mild inflammatory reactions (Bernet et al., 2004). A technical disadvantage of histopathology is its qualitative nature. For this reason, Bernet et al. (1999) have developed an evaluation scheme to transform qualitative histological observations into a semiquantitative index. In the present study, this approach was applied to study organ histopathology of brown trout from the four river basins.

The semiquantitative evaluation of liver histopathology indicated on average mild to moderate alterations in the fish populations from the various study sites, although in individual fish, much stronger pathological changes could occur. In the rivers Emme, LBK, and partly in the Necker, a downstream increase of the histopathological liver index IL was observed, although the severity of liver pathology at the downstream sites remained moderate. The downstream increase of liver integrity occurs in parallel to the increasing anthropogenic stress. A straightforward correlation of liver histopathological status to any of the abiotic, environmental parameters analysed in this study, however, could not be established. For instance, we found no consistent relation between nature and intensity of liver damage and water concentrations of nitrite or ammonia, which are known to be fish-toxic and to induce a range of liver pathological alterations (Smith and Piper, 1975; Carline et al., 1987; Lang et al., 1987; Michael et al., 1987). The failure to demonstrate a relationship between downstream impairment of liver histopathology and downstream increase of the concentrations of specific water quality parameters does not necessarily mean that water quality has no effect on liver status, since under the complex exposure situations in the field, with the presence of multiple stressors, straightforward relationships between a single stressor and a biological response may be more the exception than the rule. Establishing cause-effect relationships in field situations can be complicated even for less integrative but more agent-specific biological indices such as EROD activity. For instance, for brown trout from two moderately polluted small streams in Southern Germany, Behrens and Segner (2005) found no correlation between hepatic EROD activity and exposure to the classical EROD inducers, PHAHs and PAHs, but the best chemical predictor of EROD activity was copper. Similarly, Adams et al. (1999) reported that the EROD response of fish correlated better to environmental concentrations of metals than to PHAH levels. Although metals are not known to be able to induce EROD activity and therefore these statistical correlations obviously have no mechanistic basis, the environmental metal concentrations may be indicative of an enhanced overall contamination status including unknown EROD-inducing substances at the study sites. In this context, the importance of mixture effects for biological responses has to be emphasized, including combination of chemicals (e.g., Silva et al., 2002) as well as combinations between chemicals and physical or biological stressors. For instance, the presence of pathogens can modulate the organism response to chemicals, while chemical exposure may influence the organism resistance to pathogens (e.g. Schwaiger et al., 1997; Carlson and Zelikoff, 2002; Kiesecker, 2002).

In the case of the kidney, histopathological examinations were able to identify the presence of the parasitic disease, PKD, as a major causative factor causing downstream impairment of kidney condition. The infected kidneys displayed typical morphological characteristics of this disease such as granulomatous nephritis, accompanied by degenerative and necrotic changes of hemato-

poietic cells and of excretory renal tissue (Ellis et al., 1985). We consider the PKD infection to be responsible for the downstream increase of IK values in Emme and Venoge, since IK values of fish from the PKD-free rivers, Necker and LBK, show no significant downstream increase. It is known that PKD can result in high mortalities among brown trout populations, particularly during first infection in young-of-the-years (Hedrick et al., 1993), and population modelling has confirmed the potential adverse impact of PKD on recruitment of brown trout populations (Borsuk et al., 2006). Therefore, this disease may well contribute to the low fish biomass found at the PKD-positive downstream sites of the Emme and Venoge (Table 2). Although the results from the present study can not be conclusive with respect to the possible role of PKD in impairing brown trout populations, since only a small number of rivers/sites were studied, the fact that this disease is widespread in brown trout populations of Swiss rivers (Wahli et al., 2002; 2006), and the signifcant correlation between the abundance of young-of-the-year trout in Swiss rivers and the presence of PKD, as observed on a nation-wide basis in the project "Fischnetz" (Fischnetz, 2004) argue for PKD as a major factor in the catch decline of brown trout in Switzerland (Burkhardt-Holm et al., 2005; 2006).

Fish biometrical indices - CF, HSI – showed site-specific differences. The increase of CF values from the HS to the downstream sites, as observed at least in Emme and LBK, might be explained by a higher availability of food due to increased eutrophication and increased temperature in the lower stretches of the rivers. As long as the pollution stress is moderate, the CF values may be more dependent on the availability of nutrients in water than to water pollution status or other stress factors (Huuskonen and Lindstroem-Seppae, 1995). The same explanation as for CF may apply to the downstream increase of HSI, although the latter might also reflect an adaptive response to stress (Mayer et al., 1992). The low correlation coefficient between HSI and CF ($r = 0.3$) indicates that the two parameters are influenced by at least partly different factors.

Overall, the results from the various indices suggest that brown trout at the downstream sites, D1 and D2, show poorer health status than trout from the upstream sites, HW, although the upstream-downstream decrease is not expressed in every case, and although the magnitude of the upstream-downstream change of health indices is generally moderate. The conclusion on a decreasing health status towards the lower reaches of the four rivers is based mainly on the liver and kidney histopathological findings. Despite the fact that the four rivers differ in their hydrological, morphological and chemical parameters, the between-stream differences of the histopathological scores are moderate and rarely significant. The findings on within- and between-stream differences of health indices are fairly consistent among the two study periods, 2002 and 2003.

How does the actual fish health status correlate to the actual brown trout population status (estimated as brown trout biomass – Table 3) and the previous changes of brown trout catches in the four river systems? Changes in actual trout biomass and in actual trout health status seem to correlate at a first glance, i.e. biomass is lowest at the downstream sites where brown trout generally exhibited poorer health status. However, care must be taken not to over-interpret such relations, particularly since it is not clear which level of impairment in fish health status translates into which level of population decline, and whether the reduction of health status as observed at the downstream sites is severe enough to significantly affect population growth. Further, it must not be overlooked that there existed also several misfits between population status and health status. For instance, in the Venoge, population at the HW site showed high biomass but poor liver structure. A further caveat on over-interpreting the role of fish health in determining brown trout abundance comes from comparing fish health status in the four streams to brown trout catch decline as recorded for these rivers over the last 10 to 20 years. While magnitudes of catch decline differed from approximately 25% (Venoge) to 90% (LBK), the majority of health indices showed no significant between-stream differences, except for the PKD-associated kidney lesions. In conclusion, the results of this study point to a general association of within-stream gradients of water quality, disease (PKD) prevalence, brown trout health and brown trout biomass, however, the available database is not sufficient to establish a correlation between brown health status, as actually measured in the four rivers, and level of decline of brown trout catches as experienced during the last 10–20 years in those streams.

Acknowledgments

We thank the Cantonal Fisheries Authorities for their support in sampling the fish. Thanks go to Dr. A. Peter and Dr. E. Schager (Eawag, Kastanienbaum) for providing the population data and water temperature data. For their support in the fish sampling and field work, we are grateful to the Fisheries Authorities of the Cantones of Berne, Thurgau, Vaud, and the Principality of Liechtenstein, Dr. A. Peter and Dr. E. Schager (Eawag, Kastanienbaum), Oliver Körner, University of Basel, and Farid Firouzbakhsh (Department of Aquatic Animal Health, University of Tehran, Iran). Further, we are grateful to the Water and Soil Protection Laboratory of the Cantons Berne and Vaud and to the Environmental Protection Offices of the Canton St.Gallen and of Lichtenstein for water analysis. This study was financially supported by a grant of Hoffmann-La Roche AG Basel.

References

Adams, S. M., K. L. Shepard, J. M. S. Greeley, B. D. Jimenez, M. G. Ryon, L. R. Shugart and J. F. McCarthy, 1989. The use of bioindicators for assessing the effects of pollutant stress on fish. Mar. Environ. Res. **28:** 459–464.

Adams, S. M., M. S. Bevelhimer, M. S. Greeley, D. A. Levine and S. J. Teh, 1999. Ecological risk assessment in a large river reservoir: 6. Bioindicators of fish population health. Environ. Toxicol. Chem. **18:** 628–640. a

Altenburger, R., H. Segner and R. van der Oost, 2003. Biomarkers and PAHs – prospects for the assessment of exposure and effects in aquatic systems. In: P.E.T. Douben (ed.), PAHs: an Ecotoxicological Perspective, John Wiley & Sons Ltd., London, pp. 297–328.

Au, D. W. T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Mar. Poll. Bull. **48:** 814–834.

Behrens, A. and H. Segner, 2001. Hepatic biotransformation enzymes of fish exposed to non-point source pollution in small streams. J. Aquat. Ecosyst. Stress Recov. **8:** 281–297.

Behrens, A. and H. Segner, 2005. Cytochrome P4501A induction in brown trout exposed to small streams of an urbanized area: results of a five-year-study. Environ. Poll. **136:** 231–242.

Bernet, D., H. Schmidt, W. Meier, P. Burkhardt-Holm and T. Wahli, 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. **22:** 25–34.

Bernet, D., H. Schmidt-Posthaus, T. Wahli and P. Burkhardt-Holm, 2000. Effects of wastewater on fish health: an integrated approach to biomarker responses in brown trout (*Salmo trutta* L.). J. Aquat. Ecosyst. Recov. **8:** 143–151.

Bernet, D., H. Schmidt-Posthaus, T. Wahli and P. Burkhardt-Holm, 2004. Evaluation of two monitoring approaches to assess effects of waste water disposal on histological alterations in fish. Hydrobiologia **524:** 53–66.

Borsuk, M. E., P. Reichert, A. Peter, E. Schager and P. Burkhardt-Holm, 2006. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. Ecological Modelling, **192:** 224–244.

Broeg, K., H. v. Westernhagen, S. Zander, W. Körting and A. Koehler, 2005. The "bioeffect assessment index" (BAI). A concept for the quantification of effects of marine pollution by an integrated biomarker approach. Mar. Poll. Bull. **50:** 495–503

Burkhardt-Holm, P., A. Peter and H. Segner, 2002. Decline of fish catch in Switzerland. The project Fishnet – a balance between analysis and synthesis. Aquat. Sci. **64:** 36–54.

Burkhardt-Holm, P., W. Giger, H. Güttinger, A. Peter, K. Scheurer, M.J.F. Suter, U. Ochsenbein, H. Segner and E. Staub, 2005. Where have all the fish gone? The reasons why fish catches in Swiss rivers are declining. Environ. Sci. Technol. **39:** 441A–447A.

Burkhardt-Holm, P. and K. Scheurer, 2007. Application of weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. Aquat. Sci., **69(1):** DOI 10.1007/s00027-006-0841-6.

Carline, R. F., A. J. Benson and H. Rotenbacher, 1987. Long-term effects of treated domestic wastewater on brown trout. Water Res. **21:** 1409–1415.

Carlson, E. A. and J. T. Zelikoff, 2002. Exposure of Japanese medaka (*Oryzias latipes*) to benzo(a)pyrene suppresses immune function and host resistance against bacterial challenge. Aquat. Toxicol. **56:** 289–301.

Chavin, W., 1973. Teleostean endocrine and para-endocrine alterations of utility in environmental studies. In: W. Chavin (ed.), Responses of Fish to Environmental Change, Thomas, Springfield, IL, pp. 199–238.

Downes, B. J., L. A. Barmuta, P. G. Fairweather, D. P. Faith, M. J. Keough, P. S. Lake, B. D. Mapstone and G. P. Quinn, 2002. Monitoring Ecological Impacts, Cambridge University press, Cambridge.

Ellis, A. E., A. H. McVicar, and A. L. S. Murano, 1985. Proliferative kidney disease in brown trout, *Salmo trutta* L., and Atlantic salmon *Salmo salar* L., parr: histopathological and epidemiological observations. J. Fish Dis. **8:** 197–208.

Fairbrother, A., G. T. Ankley, L. S. Birnbaum, S. P. Bradbury, B. Francis, L. E. Gray, D. Hinton, L. I. Johnson, R. E. Peterson and G. van der Kraak, 1999. Reproductive and developmental toxicology of contaminants in oviparous animals. In: R.T. DiGiulio and D. E. Tillitt (eds.), Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates, SETAC Press, Pensacola, pp. 283–362.

Fischnetz, 2004. Final Report of Project Fischnetz (in German). Eawag, Dübendorf, Switzerland.

Götz, C., N. Chevre, H. Singer and S. Müller, 2003. Emme, Necker, Liechtensteiner Binnenkanal, Venoge: Gebietscharakterisierung, Pestizidmessungen, Toxizitätsabschätzung. Fischnetz Teilprojekt. Report-Nr. 01/15. Eawag, Dübendorf, Switzerland.

GSchV, 1998. Gewässerschutzverodnung, 1998, Bern, SR 814.201, AS 1998 2863. Council of Ministers, Switzerland.

Handy, R. D., T. Runnalls and P. M. Russell, 2002. Histopathologic biomarkers in three spined sticklebacks, *Gasterosteus aculeatus*, from several rivers in Southern England that meet the Freshwater Fisheries Directive. Ecotoxicology **11:** 467–479.

Hartmann, P. C., P. Burkhardt-Holm, W. Giger, 2006. Polybrominated diphenyl ether flame retardant in brown trout bile and liver from Swiss rivers. Environ. Poll., in press.

Hedrick, R. P., E. MacConnel, and P. de Kinkelin, 1993. Proliferative kidney disease of salmonids. Ann. Rev. Fish Dis. **3:** 277–290.

Hinton, D. E., P. C. Baumann, G. R. Gardner, W. E. Hawkins, J. D. Hendricks, R. A. Murchelano and M. S. Okihiro, 1992. Histopathologic biomarkers. In: R. J. Hugget, R. A. Kimerle, M. P. Mehrle and H. L. Bergman (eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, SETAC Publication. Lewis Publishers, pp. 155–209.

Hinton, D. E., H. Segner and T. Braunbeck, 2001. Toxic responses of the liver. In: D. Schlenk, W. H. Benson (eds.). Target Organ Toxicity in Marine and Freshwater Teleosts, Taylor & Francis, London, pp. 224–268.

Huuskonen, S. and P. Lindstroem-Seppae, 1995. Hepatic cytochrome P4501A and other biotransformation activities in perch (*Perca fluviatilis*): The effects of unbleached pulp mill effluents. Aquat. Toxicol. **31:** 27–41.

Kiesecker, J. M., 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? Proc. Natl. Acad. Sci. USA **99:** 9900–9904.

Lang, T., G. Peters, R. Hoffmann and E. Meyer, 1987. Experimental investigations on the toxicity of ammonia: effects on ventilation frequency, growth, epidermal mucous cells, and gill structure of rainbow trout, *Salmo gairdneri*. Dis. Aquat. Org. **3:** 159–165.

Mayer, F. L., D. J. Versteeg, M. J. McKee, L. C. Folmar, R. L. Graney, D. C. McCume, B. A. Rattner, 1992. Metabolic products as biomarkers. In: R. J. Huggett, R. A. Kimerly, P. M. Mehrle, H. L. Bergman (eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, Lewis Publishers, Chelsea, MI, USA, pp. 5–86.

Meyers, T. R. and J. D.Hendricks, 1985. Histopathology. In: G. M. Rand, S. R. Petrocelli (eds.), Fundamentals of Aquatic Toxicology, Hemisphere Publishing Co., New York, pp. 283–331.

Michael, M. I., A. M. Hilmy, N. A. El-Domiaty and K. Wershana, 1987. Serum transaminases activity and histopathological changes in *Clarias lazera* chronically exposed to nitrite. Comp. Biochem. Physiol. **86C:** 255–262.

Mondon, J. A., Duda, S. and B. F. Nowak, 2001. Histological, growth, and 7-ethoxy-resorufin-O-deethylase (EROD) activity responses of greenback flounder *Rhombosolea tapirina* to contaminated marine sediment and diet. Aquat. Toxicol. **54:** 231–247.

Monosson, E., 1997. Reproductive and developmental effects of contaminants in fish populations: establishing cause and effect. In: R.M. Rolland, M. Gilbertson, and R.E. Peterson (eds.),

Chemically Induced Alterations in Functional Development and Reproduction of Fishes, SETAC Press, Pensacola, pp.177–194.

Myers, M. S., L. L. Johnson, T. Hom, T. K. Collier, J. E. Stein and U. Varanasi, 1998. Toxicopathic hepatic lesions in subadult English sole (*Pleuronectes vetulus*) from Puget Sound, Washington, USA. Mar. Environ. Res. **45:** 47–67.

Rolland, R. M., 2000. Ecoepidmiology of the effects of pollution on reproduction and survival of early life stages in teleosts. Fish Fish. **1:** 41–72

Schager, E. and A. Peter, 2004. Testgebiete: Fischbestand und Lebensraum. Fischnetz Teilprojekt. Report-Nr. 00/16. Eawag, Dübendorf, Switzerland.

Scheurer, K., 2004. Zwischenbericht zu den Untersuchungen 2001–2002 in den vier Testgebieten Emme, Liechtensteiner Binnenkanal, Necker, Venoge. Fischnetz Teilprojekt, Report Nr. 00/16. Eawag, Dübendorf, Switzerland.

Schmidt-Posthaus, H., D. Bernet, T. Wahli and P. Burkhardt-Holm, 2001. Morphological organ alterations and infectious diseases in brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) exposed to polluted river water. Dis. Aquat. Org. **44:** 161–170.

Schubiger, C. (2003) Proliferative Nierenerkrankung (PKD): Diagnostische Nachweismethoden und Untersuchung krankheitsfördernder Faktoren: Doctoral Thesis, Veterinary Faculty, University of Bern, Switzerland.

Schwaiger, J., R. Wanke, S. Adam, M. Pawert, W. Honnen and R. Triebskorn, 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. J. Aquat. Ecosyst. Stress Recov. **6:** 75–86.

Schwaiger, J., 2001. Histopathological alterations and parasite infection in fish: indicators of multiple stress factors. J. Aquat. Ecosyst. Stress Recov. **8:** 231–240.

Segner, H. and T. Braunbeck, 1988. Hepatocellular adaptation to extreme nutritional conditions in ide, *Leuciscus idus melanotus* L. (Cyprinidae). A morphofunctional analysis. Fish Physiol. Biochem. **5:** 79–97.

Segner, H. and T. Braunbeck, 1990. Adaptive changes of liver composition and structure in golden ide during winter acclimatization. J. Exp. Zool. **255:** 171–185.

Segner, H. and T. Braunbeck, 1998. Cellular response profile to chemical stress. In: G. Schüürmann, B. Markert (eds.), Ecotoxicology, John Wiley, New York. pp. 521–569.

Silva, E., N. Rajapakse and A. Kortenkamp, 2002. Something from nothing – eight weak estrogenic chemicals combined at concentrations below NOEC produce significant mixture effects. Environ. Sci. Technol. **36:** 1751–1756.

Smith, C. E., R. G. Piper, 1975. Lesions associated with chronic exposure to ammonia: In: W. E. Ribelin, G. Migaki (eds.), The Pathology of Fishes, The University of Wisconsin Press, Madison, pp. 497–512.

Stebbing, A. R. D., 1985. A possible synthesis. In: B.L. Bayne (ed.), The effects of stress and pollution on marine animals, Praeger, New York, pp. 455–480.

Stehr, C. M., M. S. Myers, L. L. Johnson, S. Spencer and J. E. Stein, 2003. Toxicopathic liver lesions in English sole and chemical contaminant exposure in Vancouver Harbor, Canada. Mar. Environ. Res. **57:** 55–74.

Stentiford, G. D., M. Longshaw, B. P. Lyons, G. Jones, M. Green and S. W. Feist, 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar. Environ. Res. **55:** 137–159.

Steyermark, A. C., J. R. Spotila, D. Gilette and H. Isseroff, 1999. Biomarkers indicate health problems in brown bullheads from the industrialised Schuylkill River, Philadelphia. Trans. Amer. Fish. Soc. **128:** 328–338.

Strehler, A. , 2003. Arealstatistik and Agrarstatistik der Testgebiete von Fischnetz. Fischnetz Teilprojekt Report No. 01/16. Eawag, Dübendorf, Switzerland.

The, S. J., S. M. Adams and D. E. Hinton, 1997. Histopathological biomarkers in feral freshwater fish populations exposed to different type of contaminant stress. Aquat. Toxicol. **37:** 51–71.

Triebskorn, R., J. Böhmer, T. Braunbeck, W. Honnen, H. Köhler, R. Lehmann, A. Oberemm, J. Schwaiger, H. Segner, G. Schüürmann and W. Traunspurger, 2001. The project VALIMAR (VALIdation of biomarkers for assessment of small stream pollution): objectives, experimental design, summary of result, and recommendations for the application of biomarkers in risk assessment. J. Aquat. Ecosyst. Stress Recov. **8:** 161–178.

Van der Oost, R., J. Beyer and N. Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. **13:** 57–149.

Wahli, T., 2002. Approaches to investigate environmental impacts on fish health. Bull. Europ. Assoc. Fish Pathol. **22:** 126–132.

Wahli, T., R. Knuesel, D. Bernet, H. Segner, D. Pugovkin, P. Burkhardt-Holm, M. Escher and H. Schmidt-Posthaus, 2002. Proliferative kidney disease in Switzerland: current state of knowledge. J. Fish Dis. **25:** 491–500.

Wahli, T., D. Bernet, P. A. Steiner and H. Schmidt-Posthaus, 2007. Geographic distribution of *Tetracapsuloides bryosalmonae* infected fish in Swiss rivers: an update. Aquat. Sci., **69(1):** DOI 10.1007/s00027-006-0843-4.

Whyte, J. J., R. E. Jung, C. J. Schmitt and D. E.Tillit, 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. **30:** 347–570.